Assessment of the cytotoxic and tumor promoting potential of 2,3,7,8-TCDD and PCB congeners *in vitro*: Comparison of *in vitro*- and *in vivo*-data.

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INTRODUCTION

TCDD, PCBs and related compounds bioaccumulate as highly complex mixtures of isomers and congeners in aquatic and terrestrial food chains, thereby posing a health hazard to humans and wildlife. Among their many biologic and toxic effects, chronic toxicity is a major concern. To simplify the risk assessment of environmental contaminations, the use of toxic equivalency factors (TEFs) has been proposed[1]. The concept of TEFs is based on evidence for a common mechanism of action of TCDD-like agents via the binding to a specific receptor, the Ah receptor. TEFs can be derived from *in vivo*- or, more easily, from *in vitro*-studies, *e.g.*, the induction of CYP1A-catalyzed ethoxyresorufin O-deethylase (EROD) activity[2]. The most important limitation of this approach is that it assumes strict additivity of the TEFs of the Ah receptor agonists. However, non-TCDD-like PCBs which are quantitatively important constituents of environmental samples, show non-additive interactions with TCDD-like agonists[3]. Moreover, non-TCDD-like PCBs have the potential for chronic toxic effects, including tumor promotion[4]. Thus, there is an urgent need for further reliable *in vitro*-assays. We have, therefore, established cell culture models for the assessment of effects associated with cytotoxicity (*i.e.*, the neutral red assay) and with tumor promotion (*i.e.*, stimulation of growth and promotion of malignant cell transformation induced by initiating carcinogens). The results of these *in vitro*-studies on different PCBs (77, 118, 126 and 153) were in accord with their TEFs and their *in vivo* potencies for tumor promotion in rat liver.

MATERIALS AND METHODS

TCDD and the PCBs (77, 118, 126 and 153) were obtained from Ökometric GmbH (Bayreuth, Germany) and had a purity greater than 99%.

Hepatocytes were isolated by collagenase perfusion from adult male Wistar rats and cultured as described[5]. On the second day after cell plating, the mitotic indices were determined after 3 h exposure to colchicine (0.1 mM). Parallel cultures were used for the neutral red assay[6]. EROD activities in hepatocyte homogenates were determined fluorimetrically[7]. The transformation assay with C3H/M2 mouse fibroblasts was adjusted to determine tumor promotion according to procedures described previously[8].

RESULTS

EROD induction and cytotoxicity in primary rat hepatocytes

EROD induction was used as a standard *in vitro*-assay for TCDD-like responses. The results were in accord with those of other authors: The rank order of potencies was TCDD > PCB126 >> PCB77 > PCB118; no EROD induction was observed with PCB153 (10^{-6} M). The potencies for the induction of the cytotoxic effects were comparable to those for the induction of EROD activity. Thus, the neutral red assay is a very quick and sensitive test for TCDD- or PCB-induced cytotoxicity.

Stimulation of hepatocellular growth

The maximal enhancement of mitotic rates in primary rat hepatocytes was observed at non-toxic concentrations of TCDD (10^{-12} M) and the co-planar PCBs 126 (10^{-11} M) and 77 (10^{-9} M), *i.e.*, the growth stimulating concentrations were 100- to 1000fold lower than the maximally cytotoxic concentrations. The potencies of the PCBs 126 and 77 in relation to TCDD were about the same as their relative potencies for the induction of EROD and toxicity. PCB118 was equally potent in this assay as PCB77. PCB153 (at 10^{-8} M to 10^{-6} M) had a clear growth stimulating effect.

Promotion of malignant transformation of mouse fibroblasts

Malignant transformation of C3H fibroblasts was induced by a suboptimal dose of 3-methylcholanthrene (1 μg/ml) and further enhanced (promoted) by treatment with TCDD or PCBs. The maximally promoting concentrations of TCDD (1.5×10^{-12} M), PCB77 (3×10^{-9} M) and PCB153 (3×10^{-8} M) were about the same as those inducing hepatocellular growth.

Differential inhibition of TCDD-induced cytotoxicity and tumor promotion *in vitro*

α-Naphthoflavone (aNF), an antagonist of the Ah receptor[9], partly reduced the cytotoxic effect and completely inhibited the promoting effects of TCDD, *i.e.*, the stimulation of hepatocellular mitosis and the promotion of malignant transformation. Antioxidants (ascorbate plus α-tocopherol) and inhibitors of arachidonic acid metabolism (acetylsalicylic acid, indomethacin, caffeic acid or nordihydroguaiaretic acid) abolished the promoting effects, but were without effect on the TCDD-induced cytotoxicity *in vitro*.

Table 1: Potencies of PCB congeners relative to TCDD for tumor promotion *in vivo* and *in vitro*

PCB	*in vivo*- data		*in vitro*- data			
	TEF[a]	rPI[b]	NR[c]	EROD[c]	Mitosis[d]	Tx[d,e]
126[g]	10^{-1}	$2x10^{-2}$	10^{-1}	10^{-1}	10^{-1}	n.d.
77[h]	$5x10^{-4}$ -10^{-2}	10^{-3}	$2x10^{-3}$	10^{-3}	10^{-3}	$5x10^{-4}$
118[i]	10^{-4}	-	$2x10^{-4}$	10^{-4}	10^{-3}	n.d.
153[j]	-	$4x10^{-5}$	$<10^{-5}$[f]	$<10^{-5}$[f]	10^{-5}	$5x10^{-5}$

a) Ahlborg/WHO, 1994[1].
b) Tumor promotion indices of PCBs[4, 10] in relation to the PI of TCDD.
c) ED_{50} for TCDD/ ED_{50} for PCB.
d) ED_{100} for TCDD/ ED_{100} for PCB.
e) Tx = transformation assay; n.d. = not determined.
f) No effect up to 10^{-6} M PCB153.
g) 3,3´,4,4´,5-PCB; h) 3,3´4,4´-PCB; i) 2,3´,4,4´,5-PCB; j) 2,2´,4,4´,5,5´-PCB

DISCUSSION AND CONCLUSION

The potencies of PCBs - in relation to TCDD - for the induction of EROD activity and cytotoxicity in this *in vitro*-study were in accord with the TEFs proposed by Ahlborg et al.[1]. The potencies of the studied PCBs in the promoting assays correlated very well with their *in vivo*-potencies for tumor promotion (Table 1), based on the tumor promotion indices reported by Sargent et al.[10] and Hemming et al.[4]. Non-planar PCBs, *e.g.* PCB153, are phenobarbital-like cytochrome P450 inducers and are relatively non-toxic, but they are tumor promoters in rat liver. Similarly, in this study, PCB153 did not induce EROD acitivity and cytotoxicity, but was effective in the *in vitro*-promoting assays.

Furthermore, the mechanisms of action of TCDD/ PCBs were investigated in the *in vitro*-studies by various inhibitors. aNF exhibited only partial antagonist activity on the TCDD-induced cytotoxicity which is supposed to be mediated via the Ah receptor. Since aNF, which did not promote transformation, abolished the promoting effects of TCDD, these effects may also be Ah receptor-dependent. However, based on the studies with inhibitors of the arachidonic acid cascade and antioxidants, it is concluded that the promoting effects of TCDD involve additional mechanisms of action which are obviously not necessary for the toxic effects.

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